Thermal Destruction of *Escherichia coli* O157:H7 in Hamburger†

VIJAY K. JUNEJA,[1]* OSCAR P. SNYDER, JR.,[2] AARON C. WILLIAMS,[1] and BENNE S. MARMER[1]

[1]*U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038; and* [2]*Hospitality Institute of Technology and Management, 830 Transfer Road Suite 35, St. Paul, Minnesota 55114, USA*

(MS# 96-269: Received 27 September 1996/Accepted 20 January 1997)

ABSTRACT

The inactivation of *E. coli* O157:H7 in ground beef patties cooked in a skillet was investigated. Ground beef patties inoculated with a mixture of five strains of *E. coli* O157:H7 were cooked in a Farberware skillet set at a temperature of 275°F (137°C). Eight type K thermocouples connected to a data logger were used to record the temperatures at eight points within the patty. The cooking times studied ranged from 2.25 min to 4 min. Tryptic soy agar plates overlaid with sorbitol MacConkey agar were used for recovery of *E. coli* O157:H7. Heating of ground beef patties to an internal temperature endpoint of 155°F (68.3°C) resulted in 4-log cycle reductions of the organism. The results of this investigation conducted under conditions simulating those that occur in the retail food industry provide a basis for ensuring safety against *E. coli* O157:H7 in ground beef patties.

Key words: *E. coli* O157:H7, ground beef, heat inactivation

Escherichia coli O157:H7 continues to be recognized as a foodborne pathogen of primary concern. The organism is responsible for hemorrhagic colitis, a condition manifested by severe abdominal pain and bloody diarrhea. The disease can be followed by life-threatening complications, the most common being hemolytic uremic syndrome *(15)*. The etiology of these diseases commonly involves foodborne transmission, with ground beef being implicated often *(2, 4, 11)*. It is believed that the organism survives the cooking/grilling time and temperature applied to rare hamburgers; consumption of undercooked hamburgers contaminated with *E. coli* O157:H7 results in outbreaks of hemorrhagic colitis. Ground beef accounts for nearly one-half of all beef consumed in the United States *(9)*.

During the past years there has been substantial research pertaining to heat resistance of *E. coli* O157:H7 in ground beef using water bath and grilling methods *(5, 6, 8, 12)*. However, there is no information concerning the destruction of *E. coli* O157:H7 during cooking in a skillet. Accordingly, the objective of this study was to quantify the inactivation of *E. coli* O157:H7 in ground beef patties cooked in a skillet under conditions simulating those that occur in the retail food industry.

MATERIALS AND METHODS

Ground meat

Raw 73% lean ground beef was obtained from a local retail market and frozen (−18°C) until used (approximately 4 days). Prior to inoculation, the meat was thawed at 4°C over a 24-h period.

Bacterial strains

The five strains of *E. coli* O157:H7 used in the study were EDL-931, ent C9490 (Jack-in-the-Box), A9218-C1, 45753-35, and 933. Strains EDL-931, ent C9490, and A9218-C1 are clinical isolates and were obtained from the Centers for Disease Control, Atlanta, GA. Strains 45753-35 and 933 are meat and kidney isolates, respectively, and were obtained from the Food Safety and Inspection Service, Beltsville, MD. Individual stock cultures were maintained on Trypticase soy agar (TSA; Difco, Detroit, MI) at 4°C. The organisms were transferred periodically to maintain viability.

Preparation of test cultures

Cultures were prepared by inoculating each strain into 50 ml brain heart infusion broth (BHI) at 37°C for 24 h with two consecutive transfers. The final cultures were washed in 0.1% peptone water and centrifuged (5,000 × g, 15 min, 4°C) two times. The pellets were suspended in sterile 0.1% peptone water (wt/vol) to give a target of 8 log cells per ml of cell suspension. Equal volumes of each culture were combined in a sterile test tube to obtain a five-strain mixture of *E. coli* O157:H7 prior to inoculation of meat.

Inoculation and preparation of beef patties

A known weight of ground beef was transferred to a sterile Hobart mixer bowl and inoculated with *E. coli* O157:H7 five-strain cocktail to obtain approximately 7 log CFU/g. Inoculated meat was mixed for 2 min to ensure even distribution of the organism. Thereafter, ground beef was weighed aseptically into 100 g portions. A plastic hamburger former was used to form patties (Fig. 1). The meat was pressed and flattened with a spatula and reshaped in the patty mold. Thereafter, the meat was smoothed out so that the top was level with the plastic form. The form was pulled off to

* Author for correspondence. Tel: 215-233-6500; Fax: 215-233-6581; E-mail: vjuneja@arserrc.gov.

† Mention of brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

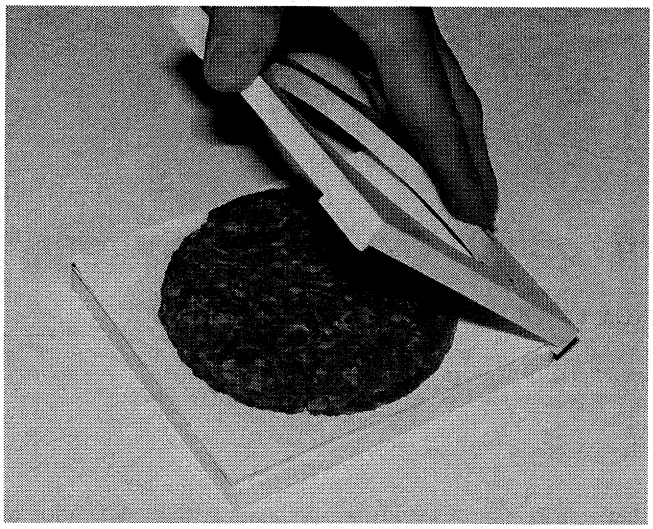

FIGURE 1. *A plastic hamburger former with a ground beef patty.*

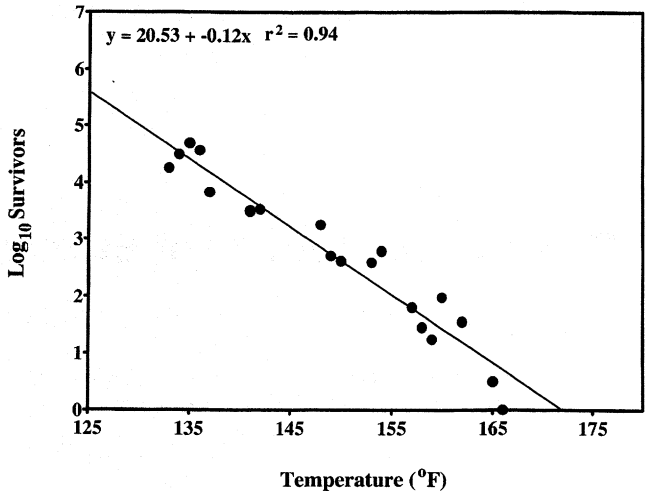

FIGURE 3. Escherichia coli *O157:H7 survivors versus internal temperature of the inoculated hamburgers. The hamburgers were cooked in a Farberware skillet set at 275°F. Each data point is the mean of two observations.*

obtain a formed hamburger patty of uniform thickness (1 cm). Patties were refrigerated (4°C) within 30 min of inoculation until used for the cooking trials (approximately 5 h). Ground beef patties not inoculated with *E. coli* O157:H7 and those inoculated but not cooked served as controls.

Cooking of hamburgers

A Farberware skillet Model 344A 1400-watt unit was used to cook the hamburger. This skillet, used to simulate a griddle, was modified with an Omega CN76000 auto-tuning thermocouple controller for temperature control. The skillet was set at a temperature of 275°F (137°C) and sprayed with a light coating of canola oil (PAM, no-stick cooking oil; American Home Foods, Madison, NJ) before cooking each hamburger. Eight type K thermocouples (Omega Engineering, Stamford, CT) were secured in an array on a utensil for mashing potatoes that was approximately the same diameter as the patties (Fig. 2). The thermocouples were attached so that when the utensil was placed on the surface of a patty, the thermocouples were positioned along radii and in the center of the patty in the thickness direction. The thermocouples were individually connected to a data logger so that the temperatures at eight points within the patty could be recorded. The thermocouple signals were sampled every 15 s, and the lowest temperature readings were considered as the internal temperature of the patty. Cooking times were varied from 2.25 min to 4 min to get a range of internal temperatures. The patties were turned every 45 s. This facilitated uniform heat penetration from both sides. As soon as the patty was turned, the potato masher temperature probe was immediately put back on the top of the hamburger. At the end of the cooking period the patties were lifted off the skillet and held for 3 min on the cooking spatula about 3 inches (ca. 7.6 cm) above the skillet before being put into a stomacher bag, plunged into a crushed ice bath, and analyzed within 30 min. This was performed to simulate the continued cooking that occurs for a short time after patties are removed from the skillet.

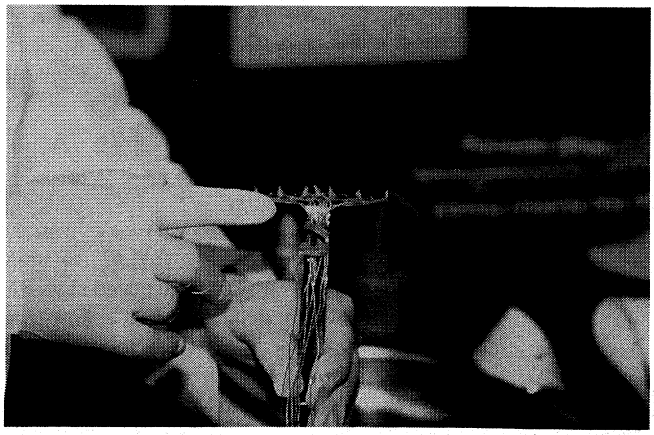

FIGURE 2. *Type K thermocouples secured in an array on a utensil for mashing potatoes.*

Microbial enumeration

Sterile 0.1% peptone was combined with each ground beef patty to obtain a 1:1 (wt/vol) slurry and homogenized for 1 min with a Stomacher Lab-blender (Model 400, Spiral Systems, Inc, Cincinnati, OH). Serial dilutions were made in 0.1% peptone followed by spiral plating of each dilution in duplicate on TSA plates using a spiral plater (Model D; Spiral Biotech, Bethesda, MD). At low bacterial levels, 0.1 ml of undiluted suspension was surface plated. In the case of inoculated beef patties, the TSA plates were overlaid with 10 ml of sorbitol MacConkey agar (pretempered to 47°C; SMA, Oxoid, Basingstoke, UK) after 2 hours of resuscitation at room temperature to allow recovery of heat-damaged cells *(10)*. All plates were incubated at 37°C for 24 h prior to counting colonies. Isolates from plates were randomly selected and subjected to serological confirmation as *E. coli* O157:H7 (RIM, *E. coli* O157:H7 Latex Test; Remel, Lenexa, KS).

RESULTS AND DISCUSSION

The present study assessed the destruction of *E. coli* O157:H7 in beef patties during cooking on a skillet set at 275°F. The results of the inoculated hamburger study are shown in Figure 3, where survivors are plotted versus endpoint temperature of the hamburger. Final temperatures of the hamburgers ranged from 133 to 166°F (56.1 to 74.4°C). The coefficient of determination (r^2) is 0.94, which shows that if the final center temperature of the hamburger is accurately determined then the actual destruction of *E. coli* O157:H7 in the hamburger patty is very predictable. The

initial starting load of the *E. coli* O157:H7 cocktail was 6.6 log CFU per g of meat. By the time the hamburger had reached 133–134°F (56.1–56.7°C), there was approximately a 2-log cycle destruction of *E. coli* O157:H7 in the hamburger. This decrease undoubtedly reflects the thermal inactivation of cells on or near the surface of the patty where temperatures capable of killing *E. coli* O157:H7 had already been reached. When the center temperature is 134°F (56.7°C), much of the outer hamburger has been cooked to a temperature well above 160°F (71.1°C), which would destroy all of the *E. coli* O157:H7 in that portion of the hamburger. The experimental data show that for every additional 9°F (5°C) increase in center temperature there was approximately an additional 1-log cycle destruction of *E. coli* O157:H7 over the range of 135 to 160°F (57.2 to 71.1°C). Thus, at an internal temperature of 155°F (68.3°C), there would be a 4-log cycle destruction of *E. coli* O157:H7 in the hamburger patty that contained 27% fat. The reductions in population densities of *E. coli* O157:H7, observed in the present study, at 155°F (68.3°C) are comparable with those made by other researchers *(6)*. Jackson et al. *(6)* reported 4.1-log cycle reductions at 155°F (68.3°C) for *E. coli* O157:H7 (rifampicin-resistant strain) inoculated into ground beef to give a final concentration of 6 log CFU/g, then the meat was refrigerated at 3°C for 9 h before grilling. Jackson et al. *(6)* quantified *E. coli* O157:H7 destruction in ground beef containing 24% fat, whereas we assessed ground beef that contained 27% fat. It is worth emphasizing that product composition affects lethality of heat to *E. coli* O157:H7 *(1)*. These authors reported that the *D* value of *E. coli* O157:H7 in ground beef heated at 131°F (55°C) in thermal death time tubes ranged from 11.4 min (beef, 7% fat) to 19.3 min (beef, 20% fat). While fat percentage in beef is not specified in the United States Department of Agriculture (USDA) regulations for cooked, uncured meat patties *(16)*, requirements include cooking temperatures and holding times ranging from 151°F (66.1°C) for at least 41 s to 157°F (69.4°C) or more for at least 10 s. In the present study, the destruction of *E. coli* O157:H7 by heating hamburgers in a skillet to an internal temperature of 155°F (68.3°C) without holding validates the cooking temperature and holding times required by the USDA to achieve log cycle reductions of the populations of *E. coli* O157:H7 that might be encountered in ground beef patties.

Previous experiments (not shown) have indicated that there are substantial differences between heating surface and product endpoint temperatures. For example, when the griddle was set at 250°F (121°C) and 350°F (177°C), the temperature endpoints at the center of hamburgers were 195°F (90.6°C) and 205°F (96.1°C), respectively. This is very reasonable, because the center of the hamburger is not heated directly by the griddle temperature, but rather by the steam-water interface temperature, which is inside the surface of the hamburger. Kotula et al. *(7)* cooked beef patties on a griddle set at 149, 177, 204, or 232°C for a total of 2 to 8 min and reported that viable coliforms were significantly ($P < 0.05$) reduced from 6 log CFU/g to less than 14 CFU/g by cooking at 177°C for 1.5 min on each side. These researchers evaluated commercial 85-g patties, whereas we evaluated 100-g patties prepared in our laboratory. It is worth mentioning that commercial patties are not uniform in consistency. Moreover, while Kotula et al. *(7)* determined the temperature of the griddle with a surface thermometer, temperature at the geometric center of the patties and at various locations in each patty was not measured to determine the location of the minimum internal temperature attained during cooking. We recorded the temperature of the patties at eight points during cooking, and the lowest temperature recorded was considered as the temperature attained. From a study similar to that of Kotula et al. *(7)*, Buck et al. *(3)* concluded that patties should be cooked at 163°C for 6 min to eliminate hazards associated with foodborne pathogens.

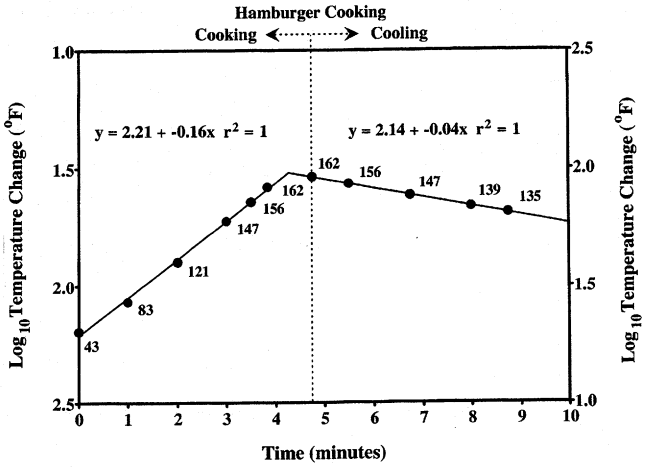

FIGURE 4. *Typical hamburger cooking and cooling process.*

The final temperature of cooking was determined from the intersection of the heating curve and the cooling curve. A typical curve is shown in Figure 4. The data are plotted according to the rules of thermal processes *(14)*. Patties continue to cook for a short time after removal from heat due to latent heat. Therefore, the cooking curve is plotted on inverted semilog paper with the top line representing a temperature 1° (F or C) below the cooking temperature. The temperature is calculated as the temperature difference between the driving force and center of the food *(14)*. In cooling, the same principles apply, except the temperature difference is plotted on an uninverted log scale with the bottom line representing a temperature 1° (F or C) above that of the cooling driving force.

A second laboratory experiment was done using typical hamburger with a moderately high spoilage aerobic plate count of 6.3 log CFU per g of beef. This was done because it is not feasible to validate hamburger cooking processes in ongoing food service operations using pathogenic bacteria. The regression plot of the endogenous aerobic bacterial flora survivors versus internal temperature of the uninoculated hamburgers is shown in Figure 5. The coefficient of determination (r^2) is 0.96. The slope of the regression line, −0.12, is similar to the slope of the line for *E. coli* O157:H7. This indicates that normal spoilage microflora in a hamburger die almost at the same rate as do the inoculated *E. coli* O157:H7 strains in the hamburger. These findings are in agreement with previous observations reported by other researchers *(3)*. Buck et al. *(3)* cooked ground beef patties on a grill at 137°C

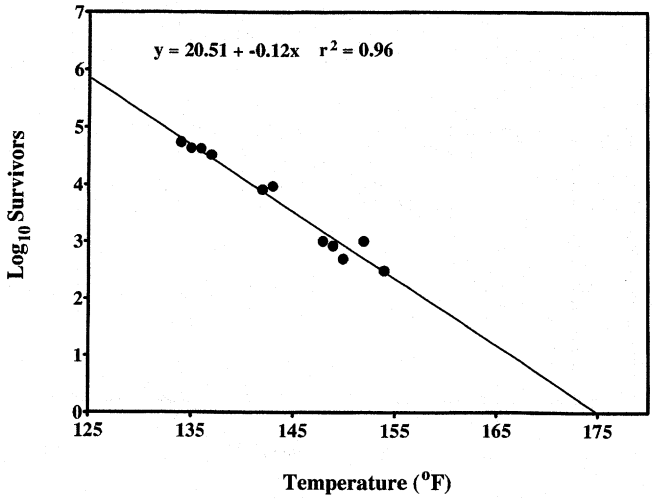

FIGURE 5. *Endogenous bacterial flora survivors versus internal temperature of the uninoculated hamburgers. The hamburgers were cooked in a Farberware skillet set at 275°F. Each data point is the mean of two observations.*

and reported that bacterial population reductions (3 log within 6 min) were similar in total aerobic bacteria and in *E. coli.* In the present study, a temperature increase of 8.5°F (4.7°C) was sufficient to reduce the normal spoilage flora population by 1 log over the range of 135 to 154°F (57.2 to 67.8°C).

These experiments have shown that the destruction of *E. coli* O157:H7 or spoilage bacteria in a hamburger can be predicted if the hamburger is cooked in a way so that the center of the hamburger is measured as a cold spot. This way, the temperature can be measured very closely with a precise thermocouple. Actually, what is being measured when the microorganisms are recovered in the hamburger is the integrated lethality associated with destruction of the bacteria throughout the hamburger patty. The patty on the skillet does not cook uniformly in a given time at a given temperature, as it would in a water bath, but is cooked from the outside toward the center, where the center volume contains the surviving organisms.

The finding that the spoilage microflora die almost the same as the *E. coli* O157:H7 is also very important in that the present approach provides a simple way, without introducing pathogens in a retail food operation, to develop a process authority to validate the safety of a cooking process in the retail food operation. The key to obtaining these results is the correct use of a precise thermocouple. Note that any other form of temperature measuring device, such as a bimetallic coil thermometer, is not sufficiently accurate or rapid to control the safe cooking of hamburgers and thin foods *(13).*

CONCLUSIONS

This study has shown that a five-strain cocktail of *E. coli* O157:H7 can be controlled successfully in a retail food operation or the home, as long as a critical control point temperature measurement technique is used in cooking and correct procedures are used for monitoring. It is expected that this finding will apply to all forms of meat being cooked on grills and griddles, or foods fried in deep-fat fryers. If the cook can find the coldest temperature in a food, the safety of the food will be very predictable. Our results indicated that if ground beef (73% lean) patties are cooked to an internal temperature of 155°F (68.3°C), there would be a 4 log reduction of *E. coli* O157:H7. However, the influence of meat composition (fat and moisture content, content of extenders) on the lethality of *E. coli* O157:H7 during cooking of beef patties in a skillet warrants further investigation. Also, the effect of heating rate in development of thermotolerance and the time and temperature necessary for inactivation of *E. coli* O157:H7 in hamburger need to be defined.

REFERENCES

1. Ahmed, M. N., D. E. Conner, and D. L. Huffman. 1995. Heat-resistance of *Escherichia coli* O157:H7 in meat and poultry as affected by product composition. J. Food Sci. 60:606–610.
2. Belongia, E. A., K. L. MacDonald, G. L. Parham, K. E. White, J. A. Korlath, M. N. Lobato, S. M. Strand, K. A. Kasale, and M. T. Osterholm. 1991. An outbreak of *Escherichia coli* O157:H7 colitis associated with consumption of precooked meat patties. J. Infect. Dis. 164:338–343.
3. Buck, C. B., D. Montgomery, and D. B. Pratt. 1975. The effect of cooking on the quantity of *Escherichia coli* in ground beef. Res. Life Sci., U. of Maine 23(2):6–9.
4. Doyle, M. P. 1991. *Escherichia coli* O157:H7 and its significance in foods. Int. J. Food Microbiol. 12:289–302.
5. Doyle, M. P., and J. L. Schoeni. 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. Appl. Environ. Microbiol. 48:855–856.
6. Jackson, C. J., M. D. Hardin, and G. R. Acuff. 1996. Heat resistance of *Escherichia coli* O157:H7 in a nutrient medium and in ground beef patties as influenced by storage and holding temperatures. J. Food Prot. 59:230–237.
7. Kotula, A. W., C. M. Chesnut, B. S. Emswiler, and E. P. Young. 1977. Destruction of bacteria in beef patties by cooking. J. Anim. Sci. 45:55–58.
8. Line, J. E., A. R. Fain, A. B. Mogan, L. M. Martin, R. V. Lechowich, J. M. Carosella, and W. L. Brown. 1991. Lethality of heat to *Escherichia coli* O157:H7: D-value and z-value determination in ground beef. J. Food Prot. 54:762–766.
9. Marriott, N. G., R. A. Garcia, J. H. Pullen, and D. R. Lee. 1980. Effect of thaw conditions on ground beef. J. Food Prot. 43:180–184.
10. McCleery, D. R., and M. T. Rowe. 1995. Development of a selective plating technique for the recovery of *Escherichia coli* O157:H7 after heat stress. Lett. Appl. Microbiol. 21:252–256.
11. Riley, L. W. 1987. The epidemiologic, clinical, and microbiologic features of haemorrhagic colitis. Annu. Rev. Microbiol. 41:383–407.
12. Shipp, D. K., T. G. Rehberger, and L. W. Hand. 1991. Thermal resistance of *Escherichia coli* O157:H7 in ground beef patties, p. 92–97. *In* 1991 Animal science research report. Oklahoma Agricultural Experiment Station, Stillwater, OK.
13. Synder, O. P. 1996. Limitations of bimetallic-coil thermometers in monitoring food safety in retail food operations. Dairy Food Environ. Sanit. 16:300–304.
14. Stumbo, C. R. 1965. Thermobacteriology in Food Processing. Academic Press, Inc. New York.
15. Tarr, P. I. 1994. Review of 1993 *Escherichia coli* O157:H7 outbreak: western United States. Dairy Food Environ. Sanit. 14:372–373.
16. United States Department of Agriculture. 1996. Heat processing procedures, cooking instructions, and cooling, handling and storage requirements for uncured meat patties. 9CFR 318.23. Office of Federal Register, National Archives and Records Administration, Washington, D.C.